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Methods of Breeding Chelonus annulipes on the Mediterranean Flour Moth for Use Against the European Corn Borer

By WILLIAM G. BRADLEY, *associate entomologist, Division of Cereal and Forage Insect Investigations, Bureau of Entomology and Plant Quarantine* [1]

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INTRODUCTION

Chelonus annulipes Wesm.[2] is a parasite of the European corn borer (*Pyrausta nubilalis* (Hbn.)) native to northern Italy and first imported into the United States in 1929. Details of the biology and morphology of this parasite have been published by Vance,[3] and discussions of various phases of its physiology have been given by Wishart and Van Steenburgh [4] and by Bradley and Arbuthnot.[5] Its biology is briefly summarized as follows: The female deposits her egg in that of the host; the parasite larva has at least three instars within the host larva, and during the last instar issues from the host, feeds externally until all remaining contents of the latter are devoured, and spins a cocoon for pupation. This bulletin describes the methods developed

[1] The writer acknowledges his indebtedness to W. A. Baker, whose suggestions and criticisms of the manuscript are much appreciated, and to E. W. Beck for his helpful association and for furnishing photographs used in figures 4, 6, 7, 11, and 12.

[2] Order Hymenoptera, family Braconidae.

[3] VANCE, ARLO M. THE BIOLOGY AND MORPHOLOGY OF THE BRACONID CHELONUS ANNULIPES WESM., A PARASITE OF THE EUROPEAN CORN BORER. U. S. Dept. Agr. Tech. Bul. 294, 48 pp., illus. 1932.

[4] WISHART, GEO., and VAN STEENBURGH, W. E. A CONTRIBUTION TO THE TECHNIQUE FOR PROPAGATION OF CHELONUS ANNULIPES WESM., AN IMPORTED PARASITE OF THE EUROPEAN CORN BORER. Canad. Ent. 66: 121–125, illus. 1934.

[5] BRADLEY, W. G., and ARBUTHNOT, K. D. THE RELATION OF HOST PHYSIOLOGY TO DEVELOPMENT OF THE BRACONID PARASITE, CHELONUS ANNULIPES WESMAEL. Ent. Soc. Amer. Ann. 31: 359–365, illus. 1938.

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for breeding this parasite for colonization in the United States as an aid to the biological control of the European corn borer. The work was done at the Toledo, Ohio, laboratory of the Bureau of Entomolgy and Plant Quarantine.

EARLY RELEASES

Releases of *Chelonus annulipes* in southeastern Massachusetts and eastern Rhode Island were made each year during the period 1929 to 1933, inclusive. The parasites for these releases were obtained from northern Italy and by breeding, with corn borer larvae as host. In the fall of 1932 one specimen was taken east of the release point at East Providence, R. I. In the spring of 1933 another specimen was recovered near the Bridgewater, Mass., release point. In the fall of 1934 several specimens were collected near Dighton, Mass., and collections made in the fall of 1935 showed that the parasite was maintaining itself in encouraging numbers at this point. This discovery indicated the desirability of further distribution of the parasite throughout the eastern area of corn-borer infestation. As at this time the successful rearing of *Chelonus blackburni* Cam. on the Mediterranean flour moth (*Ephestia kuehniella* Zell.) had been accomplished by Noble and Hunt [6] at Presidio, Tex., an attempt was made to rear *C. annulipes* on the same host.[7]

PRELIMINARY EXPERIMENTS

At the beginning of this work the possibility of rearing *Chelonus annulipes* on eggs of the Angoumois grain moth (*Sitotroga cerealella* (Oliv.)) was considered. Through W. H. Britton, of the Connecticut Agricultural Experiment Station, several cards of eggs of *S. cerealella* were obtained. At the time when one female *Chelonus* was steadily ovipositing on eggs of *Ephestia kuehniella* in a vial cage, a piece of a card containing eggs of *S. cerealella* was inserted into the oviposition vial and the card of *Ephestia* eggs quickly removed. The female immediately ceased ovipositing and, after the first touch with her antennae, paid no more attention to the *Sitotroga* eggs. The *Sitotroga* eggs were then quickly removed and replaced with *Ephestia* eggs. The female resumed ovipositing at once. As these *Sitotroga* eggs had been treated, on their receipt, with carbon disulfide to control an infestation of mites, it is possible that they had not been aired a sufficient length of time to prevent a deterrent effect from the odor of the carbon disulfide.

Through the courtesy of the Entomological Branch of the Canadian Department of Agriculture a few mated females of *Chelonus annulipes* were obtained in September 1936. A supply of a rapidly developing genetic strain of the Mediterranean flour moth in various stages of development was then obtained at a local feed mill, and experiments were carried on from September 1936 to March 1937 to determine the most efficient technique for rearing both host and parasites in large

[6] NOBLE, L. W., and HUNT, W. T. IMPORTED PARASITES OF PINK BOLLWORM AT PRESIDIO, TEX., 1932–36. Jour. Econ. Ent. 30 : 842–844. 1937.

[7] K. A. Bartlett later was successful in rearing *Chelonus annulipes* on *Diatraea saccharalis* (F.) and *Corcyra cephalonica* (Staint.) in Puerto Rico. (Unpublished data received from Dr. Bartlett in 1938.)

quantities. On completion of these preliminary experiments the parasite material originating from adults bred in Canada was discarded. In June 1937 a fresh supply of mated females from material imported from Italy that season was obtained from Moorestown, N. J., so that the experiments might be continued and a stock maintained until time for mass rearing for release in 1938.

HOST OVIPOSITION

Waxed, cylindrical, cardboard food cartons of ½-pint capacity were tried in various ways for confining the host adults during their oviposition period. The method finally adopted was as follows:

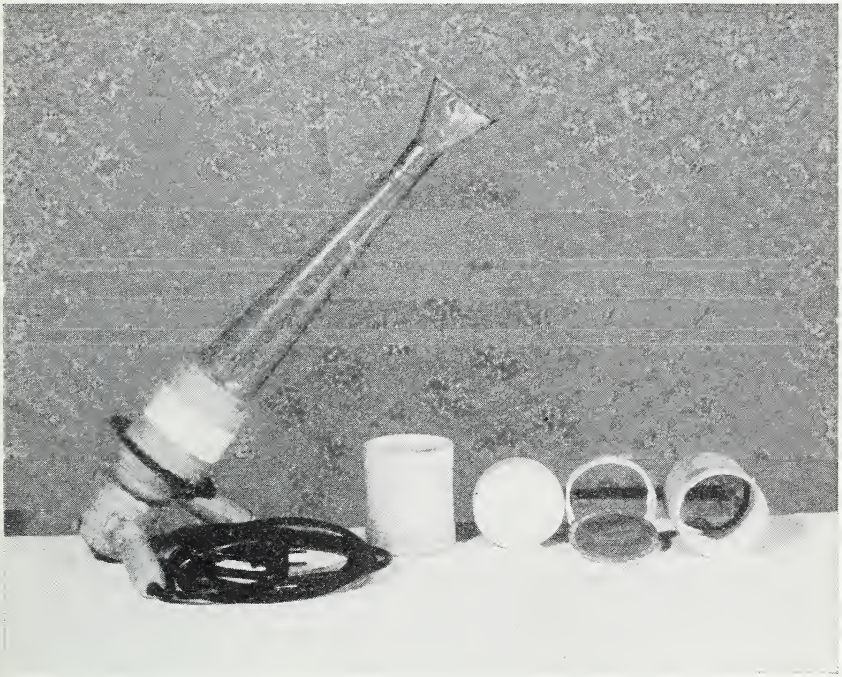

FIGURE 1.—Apparatus devised for collecting host moths and cartons used for confining the moths during oviposition.

The top of the cover and bottom of the container were removed and in their places were substituted disks of 18-mesh copper screen, reinforced by narrow flat rings of zinc soldered around their peripheries. Each carton, when stocked with adults, was set into a cover from another carton into which was sifted a small quantity of flour. Thus, during oviposition, the adults were confined in a screen-top cage having a screened bottom through which the eggs were deposited into the flour below (fig. 1). Fine bolted white flour was used for all host oviposition in order to facilitate the separation process to follow.

It was noted that the moths would deposit a considerable number of eggs in cages under which no flour was used. Therefore, with a view to eliminating several operations necessitated by the use of flour,

6 lots of 100 *Ephestia* adults each were caged in oviposition cartons, 3 of the lots being supplied with flour and 3 being without flour. The adults were selected at random for all cages. Oviposition continued through 4 days. Of the total number of eggs (30,129) laid by the 600 adults in the 4 days, 65.24 percent were laid by those which were supplied with flour. As this was almost twice the number laid by those ovipositing without flour, it was concluded that the presence of flour had a favorable influence on egg production.

It was also noted that in the case of the three lots of moths ovipositing in flour 31 percent of their eggs were laid during the first 24 hours after collection (moth collections in the emergence cages were made daily), 29 percent during the second day, 26 during the third, and 14 during the fourth day. In the case of the three lots not supplied with flour, representing about one-third the total eggs laid by the two groups, 24 percent of their eggs were laid the first day, 33 the second, 31 on the third, and 11 on the fourth day.

During the preliminary experimental work a considerable number of the host eggs had failed to hatch, and it was thought that this might be partly due to infertility. In view of the observation that absence of flour apparently retarded oviposition activities, it was thought that this longer opportunity for the females to mate before egg deposition might result in higher egg fertility. To test this point 6 lots of 50 eggs each from each of 4 days' oviposition were observed for hatching. Three of the lots of eggs from each day's oviposition were from adults provided with flour and three from adults not supplied with flour. Table 1 shows the results of this test.

TABLE 1.—*Comparison of percentages of hatch of eggs laid in the presence of flour with those of eggs laid in its absence*

Lot No.	Hatch of eggs laid during the day indicated with—							
	Flour supplied				No flour supplied			
	First	Second	Third	Fourth	First	Second	Third	Fourth
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
1	60	58	38	40	64	42	12	26
2	56	72	42	40	60	58	26	24
3	66	64	48	44	64	54	30	24
Average	60.7	64.7	42.7	41.3	62.7	51.3	22.7	24.7

It may be noted that there was no great difference in hatch between the eggs laid in the flour and those laid in its absence during the first 2 days. The decrease in the percentage of hatch of eggs in both lots laid after the second day indicated the possibility of increasing production of parasites by using for parasitization only those eggs deposited during the first 2 days after emergence and most likely to produce host larvae.

To test further whether lack of opportunity to mate was responsible for some of the failure to hatch, the 4-day egg production from 5 lots of adults was compared. The adults in 3 of these lots were collectel in the normal manner from the emergence cage; that is, single males, single females, and mating pairs were collected at random as they rested on the sides and roof of the cage. No attempt was made to

select individuals of one class in preference to those of any other nor was the number of individuals noted. As the adults in the remaining 2 lots were collected in copula, 50 percent in these lots were females and all were mated. The lots in which the adults were collected at random produced 66 eggs per adult whereas the lots composed of mated pairs produced 68 eggs per adult, an insignificant variation. Furthermore, no increase in percentage of hatch was noted for eggs laid by the females known to have mated. This test indicated that when adults were collected at random from the emergence cages, nearly equal proportions of males and females were obtained, and that females so collected were mated as well as those actually collected in copula.

In other experiments it was found that there was no decrease in the numbers of eggs laid per adult as the numbers of moths were increased per carton up to 150; in fact, a slight amount of crowding seemed to increase egg production. As the density of the population in the cartons was increased beyond 150 adults per carton, the egg production per female decreased but the egg production per carton increased up to a limit of about 500 adults per carton. Since host adults were usually plentiful during the breeding work, the smallest number of cartons necessary to supply the number of eggs desired daily was utilized. No water was furnished the adults in the oviposition cartons since it was found that such deprivation did not affect egg deposition. This substantiated the findings of Norris,[8] that although other members of the genus *Ephestia* were benefited by having access to water, *Ephestia kuehniella* was affected only slightly. Norris also found that by feeding the females sugar, longevity was increased but fecundity was affected only slightly.

Oviposition occurred for the most part without the use of lights, since Norris had shown that the fecundity of females is not affected by keeping them in total darkness throughout adult life.[8] In all experiments in which careful records were kept, an average of 161 eggs per female was obtained.

The screened cartons were left standing in the inverted covers for 24 hours. At the end of this period clean covers and fresh flour were substituted. This procedure was followed daily through 4 days with each carton. Such a small proportion of the eggs was laid after the end of the fourth day that the contents of all cartons were discarded then.

With this equipment, as with all others, a paramount consideration was the prevention, as far as possible, of contamination of the material with disease and mites. The screen tops and bottoms as well as the covers were removable. After their contents were discarded at the end of the fourth day all cartons were disassembled and the various parts washed in alcohol prior to their continued use.

No trouble with mites or disease was experienced during any of the work with *Ephestia* at the Toledo laboratory. In order to be prepared in the event of an infestation by mites, however, tests were conducted to determine the effect of treating host eggs with carbon disulfide. Eggs attached to cards were immersed in carbon disulfide

[8] NORRIS, MAUD J. CONTRIBUTIONS TOWARDS THE STUDY OF INSECT FERTILITY.—II. EXPERIMENTS ON THE FACTORS INFLUENCING FERTILITY IN EPHESTIA KÜHNIELLA Z. (LEPIDOPTERA, PHYCITIDAE). [London] Zool. Soc. Proc. 1933: 903–934. 1933. (See p. 914.)

——— CONTRIBUTIONS TOWARDS THE STUDY OF INSECT FERTILITY.—III. ADULT NUTRITION, FECUNDITY, AND LONGEVITY IN THE GENUS EPHESTIA (LEPIDOPTERA, PHYCITIDAE). [London] Zool. Soc. Proc. 1934: 344–360. 1934. (See pp. 344 and 349.)

and allowed to dry. The treatment had no effect on the viability of the eggs, but the parasite adults oviposited slightly less readily in treated than in untreated eggs. It is probable that by allowing a longer time for all odor to leave the eggs this difficulty could be entirely avoided.

CLEANING AND HANDLING HOST EGGS

To separate the host eggs from the flour particles with which they were intermingled, a funnel containing a screen of 80-mesh copper wire was used. The covers containing the host eggs laid during the

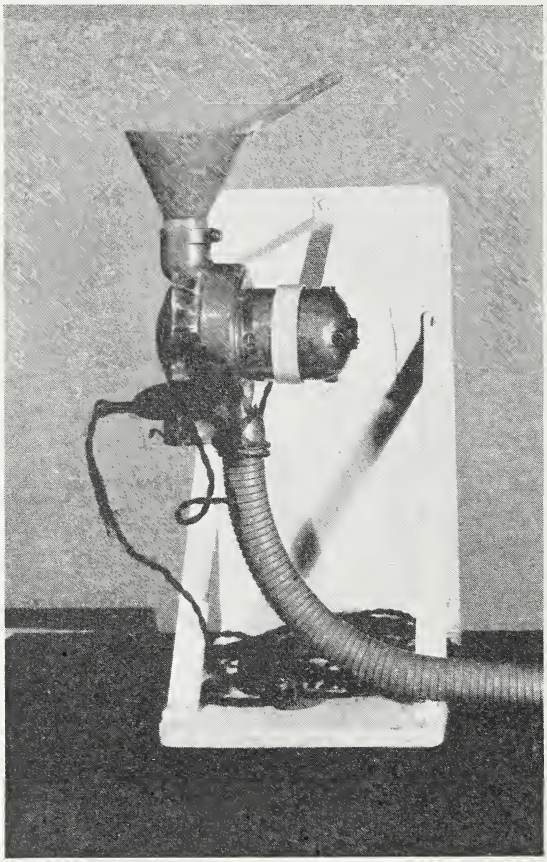

FIGURE 2.—Apparatus for separating eggs from the flour, adapted for use with a vacuum cleaner.

previous 24 hours were inverted over the funnel and their contents brushed over the 80-mesh screen. The outlet of the funnel was fitted into the intake tube of a vacuum cleaner converted to this use (fig. 2). The suction was so regulated that the eggs were not injured by the air currents, whereas the flour was drawn through the screen when brushed over it with a circular motion by a soft brush. When large particles of other matter, such as parts of moth wings and legs, were present, they were first removed by passage of the material through a sieve of 40-mesh screen.

After the host eggs had been separated from extraneous matter, they were fastened to cards in clusters. It was found that when they were scattered on cardboard moistened with water the natural secretions on the chorion of the eggs caused them to adhere firmly to the card on drying. To obtain the desired number of eggs in each cluster, a metal pattern was made having circular holes of such size that when the eggs were scattered over it evenly about 100 eggs would go through each hole onto the moistened card below. The cards used were 3 by 5 inches, and 28 clusters of approximately 100 eggs each were put on each card (fig. 3). Each day's production of host eggs was weighed and the number available ascertained, as 1,000 eggs weighed 0.03 gram. Allowing 2,800 eggs per card, the number of cards that could be filled each day was ascertained, and the eggs

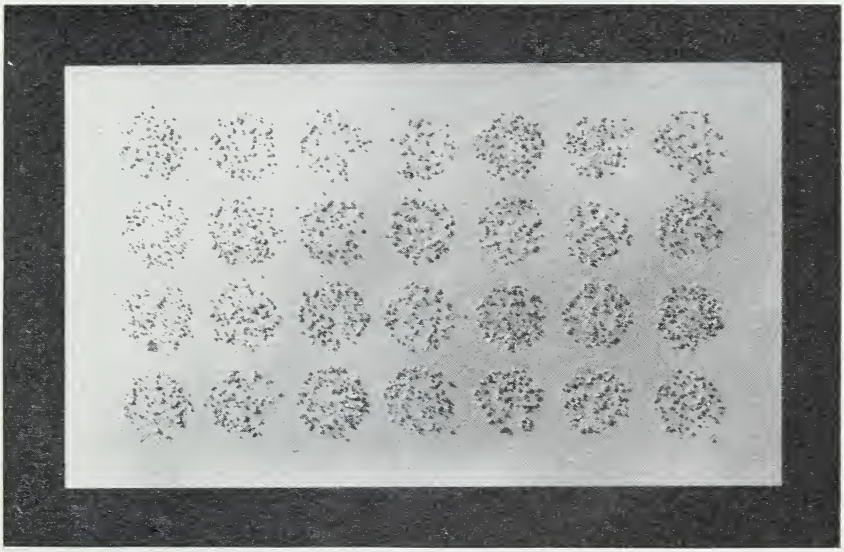

FIGURE 3.—Clusters of approximately 100 eggs of *Ephestia kuehniella* attached to a 3- by 5-inch card.

were distributed uniformly over them. Check counts from time to time showed that while there was some range in the number of eggs in different clusters, the average number per cluster was approximately 100. Eggs were cleaned of flour directly after being taken from the incubator room. They were then immediately poured into a common salt shaker (fig. 4) which was used to scatter them over the cards.

Host eggs were not exposed to the parasites until the day after they had been cleaned and were stored during this interval on the shelves of a small metal rack (fig. 4) in a covered earthenware crock. The age of the eggs at the time of parasitization was therefore between 24 and 48 hours. Various methods of holding the eggs through the 24-hour period elapsing between their removal from the incubator room and their presentation for parasitization (such as keeping them covered with flour, scattering them on cards at the end of, instead of preceding.

the 24-hour interval, holding them at room temperature and humidity, and holding them in closed containers at a high humidity) were tried, but no method proved more satisfactory than that outlined above.

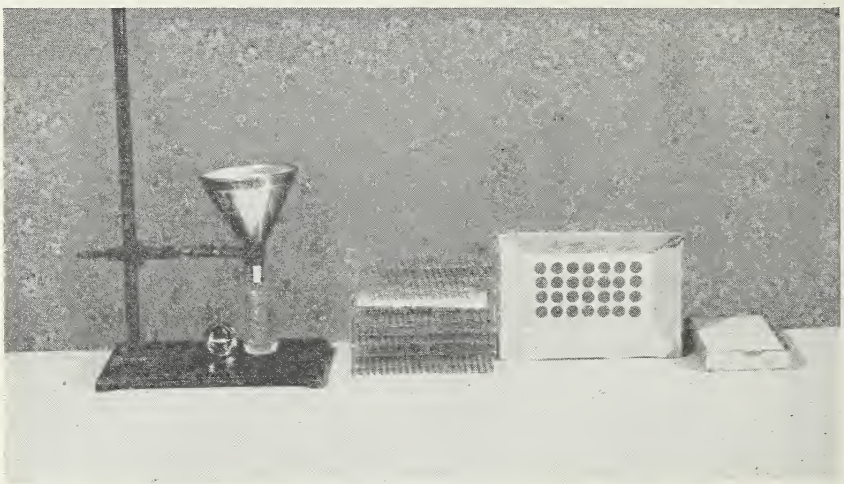

FIGURE 4.—Salt shaker used for distributing the host eggs, metal pattern for forming the clusters on the cards, and screen-wire drying rack.

PARASITE OVIPOSITION

From previous experience in rearing *Chelonus annulipes* on European corn borer eggs it was known that a greater percentage of parasitized eggs were obtained when the parasite females were isolated during oviposition than when a number were confined in a single cage.

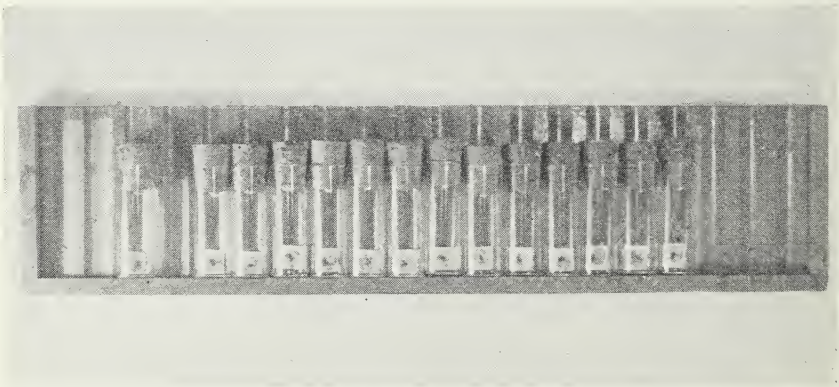

FIGURE 5.—Parasite oviposition vials showing females ovipositing in host eggs in clusters cut from the cards.

This increased production outweighed the saving in time and labor offered by the mass oviposition method. Assuming that this would be the case with *Chelonus* ovipositing in eggs of the Mediterranean flour moth, the parasite females, after they had mated, were isolated with a cluster of eggs in 3- by 1-inch cork-stoppered shell vials (fig. 5), in

which they remained throughout the oviposition period. Food was supplied in the form of an agar-sugar drop [9] on a piece of paper pinned to the inner end of the cork. At 3-day intervals the agar-sugar was moistened with a drop of water.

Before the mated parasite females were brought from their storage quarters, each card bearing the host eggs was cut into 28 portions, with 1 cluster on each portion. On being brought into the parasite oviposition room the vials containing the mated females were placed with their bottoms toward the light source. One cluster of host eggs was then put in each vial near its bottom with the eggs uppermost on the card (fig. 5). The vials were watched and only those clusters in which the females were noted to have actually attempted oviposition were used for rearing. At the end of an interval of 1 to 2 hours the females lost interest in the eggs. The cards were then removed; and at this time, if needed, moisture was applied to the agar-sugar drop and the vial placed with the cork stopper toward the light source so that feeding might occupy the attention of the parasites during the period in which the cards were being removed.

When the females were not ovipositing, they were kept in the dark at a temperature of 58° to 60° F. They were brought out for daily oviposition periods. This technique in handling the adults resulted in extreme longevity, some of the females living for approximately 2 months.

To save material and labor in maintaining hosts and parasites, it was desirable that each be reared in numbers just sufficient to maintain the stock safely. After some experimentation it was found that 100 eggs supplied to each female daily permitted unrestricted parasitization and the survival of enough unparasitized eggs to maintain the host stock.

REARING OF HOST LARVAE

In the preliminary tests in rearing flour moth larvae it was noted that, regardless of the depth of the flour in the rearing box, the larvae seldom penetrated deeper than one-fourth of an inch. This suggested the use of shallow rearing trays; and since the danger of epidemics of disease and mites made small units desirable, boxes with covers, made of 0.052-gage newsboard, 6 inches square and 1 inch deep, were finally utilized (fig. 6). Cardboard was selected because it was found that the water of metabolism given off by large numbers of larvae caused mold in other containers made of less porous or less absorbent material. Richardson [10] states that the larvae of *Ephestia kuehniella* are affected by a protozoan parasite which often causes a heavy mortality and may at times kill all the larvae in a bottle. In the cardboard rearing boxes a psocid, *Liposcelis divinatorius* (Müll.), was usually present but never increased in numbers sufficient to cause inconvenience.

A review of the literature indicated that whole-wheat flour promoted more rapid growth and produced healthier and larger host larvae and greater fecundity in the adults than any other food. This

[9] HOLLOWAY, J. K. AN AGAR-SUGAR PREPARATION USED AS FOOD IN PARASITE SHIPPING CAGES. U. S. Bur. Ent. and Plant Quar. Cir. ET–91, 1 p. 1936. [Mimeographed.]

[10] RICHARDSON, CHARLES H. A PHYSIOLOGICAL STUDY OF THE GROWTH OF THE MEDITERRANEAN FLOUR MOTH (EPHESTIA KUEHNIELLA ZELLER) IN WHEAT FLOUR. Jour. Agr. Res. 32: 895–929, illus. 1926.

material was therefore used. To avoid mite contamination and the necessity for fumigation, it was always obtained in small, freshly ground lots from a local feed mill.

The whole-wheat flour was spread in an even layer one-fourth of an inch in depth over the bottom of each box. This layer required about 120 cubic centimeters of flour. The optimum number of eggs with which to infest this quantity of flour was the subject of experimentation. Norris had already shown that between 0.125 and 0.15 gram of whole-wheat flour was required by a larva subsequently developing into a female possessing full potential fecundity,[11] and in the present experiments it was found that the maximum hatch obtained in mass rearing did not exceed 50 percent, principally because of sterility. Allowing 0.15 gram per larva, the 120 cubic centimeters

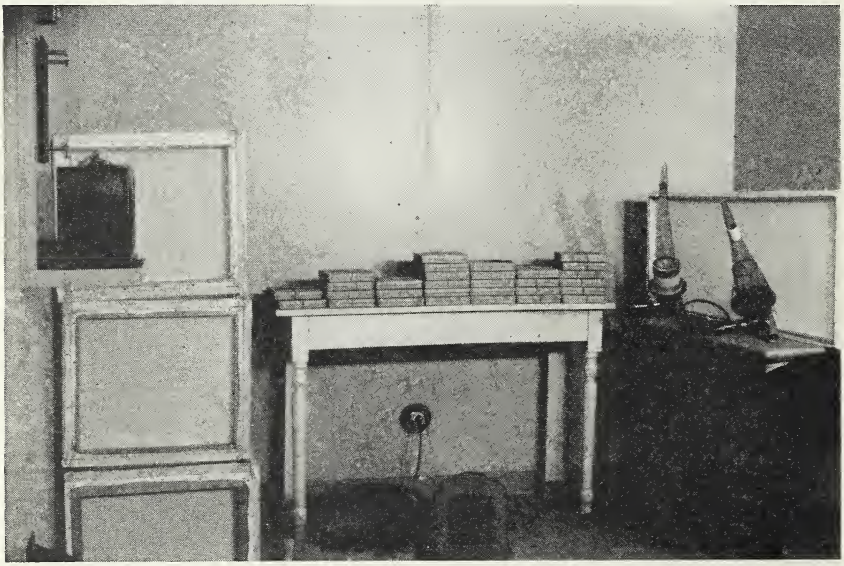

FIGURE 6.—Incubator room showing emergence cages and rearing boxes used for rearing *Ephestia kuehniella* for parasitization of its eggs.

of flour would support 800 larvae and permit the use of 1,600 eggs. Actually, 1,500 eggs, or 15 clusters as they were taken from the parasite oviposition vials, were allocated to each rearing box. When larger numbers were used, the entire quantity of flour was consumed before the majority of the larvae had matured. When smaller quantities of eggs were used, a considerable quantity of unconsumed flour remained in the boxes after adult emergence. It was noted also that consumption of flour rapidly accelerated after the twenty-first day. Accordingly, a thin layer of whole-wheat flour was scattered over the webbing in the box at this time.

All rearing and host oviposition were conducted in an incubator room, measuring 15½ by 8 by 6½ feet, with controls set to keep the temperature at 80° F. and to prevent the relative humidity from going below 70 percent. While large numbers of parasites were be-

[11] See p. 912 of the article referred to in footnote 8 (p. 5).

ing reared for liberation, however, and when about a quarter of a million host larvae were in the room, the moisture given off by their metabolic functions was sufficient to raise the relative humidity to about 95 percent and to hold it there. As the relative humidity of the air outside the incubator room at this time averaged about 60 percent, the extent of the humidifying action of the growing larvae can be seen. No tests were directed specifically toward finding the optimum humidity. Observations, however, seemed to show that this high humidity favored the development of the host although a lower humidity would have been better for parasite production.

EMERGENCE OF PARASITE AND HOST ADULTS

Both host and parasite emergence began about 32 days after parasitization of the host eggs. In general, at the end of 30 days most feeding had ceased and host and parasite cocoons were numer-

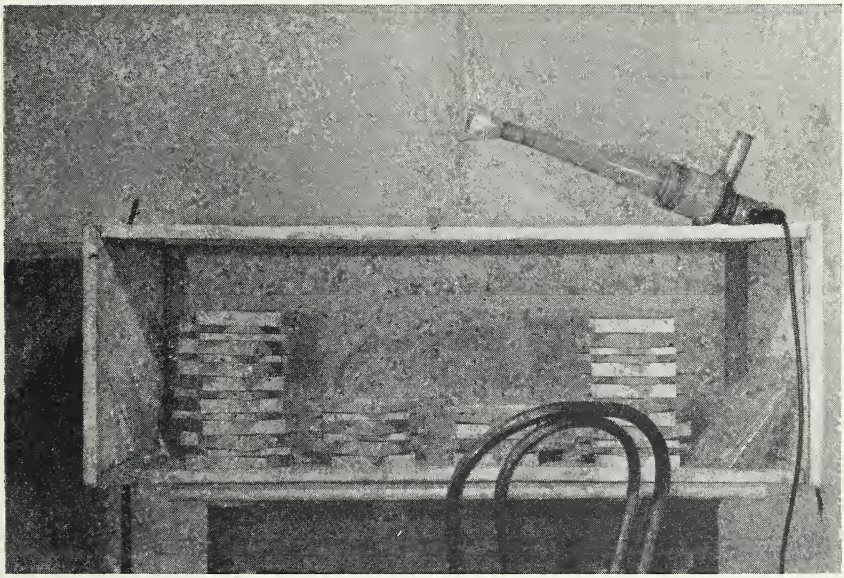

FIGURE 7.—Emergence cage showing cardboard rearing boxes stacked to permit adult emergence.

ous on the covers and sides of the boxes. Accordingly, after a rearing interval of 1 month the covers of the boxes were removed, inverted beneath the box, and stacked crosswise of one another in an emergence cage so as to allow egress of the host and parasite adults (fig. 7).

Emergence of parasite adults and host moths was closely synchronized. The average time from egg deposition to emergence of the adult in the case of *Chelonus annulipes*, when reared on *Ephestia kuehniella* at a constant temperature of 80° F. and high relative humidity, was found to be 44.85 days. This mean was obtained from observations on 4 generations of parasites, comprising over 140,000 individuals reared from June to December in 1937 and 1938. The

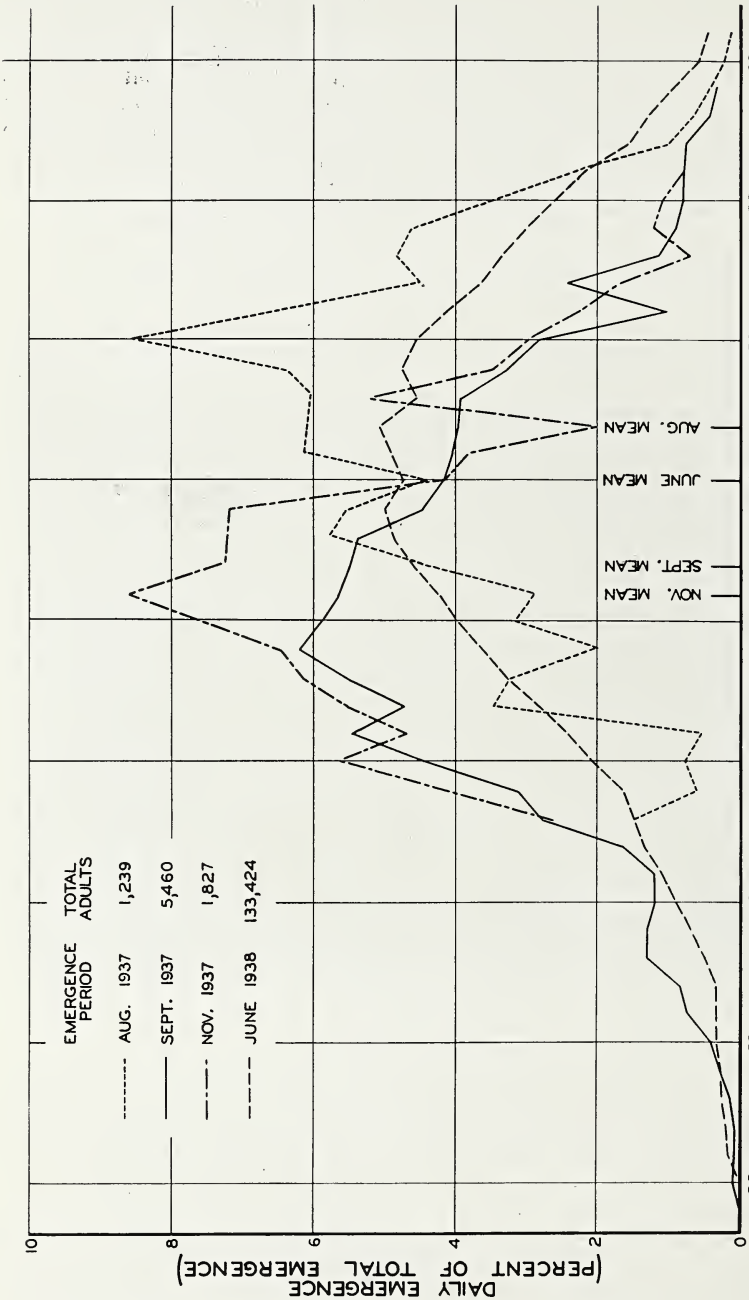

FIGURE 8.—Rate of development of *Chelonus annulipes* counting from the middle of the oviposition period. Toledo, Ohio, 1937 and 1938.

average length of the life cycle for each generation was considered as the number of days intervening between the date when 50 percent of the parasite eggs had been deposited and the date when 50 percent of the parasite adults had emerged.

Records on host emergence were not taken; but since the flour moth eggs were laid during approximately the same period as the parasite eggs, and since moth emergence began and ended synchronously with emergence of the parasites, the life cycle of the host closely approximated that of the parasite.

Figure 8 shows the rate of development of parasites reared at the Toledo laboratory.

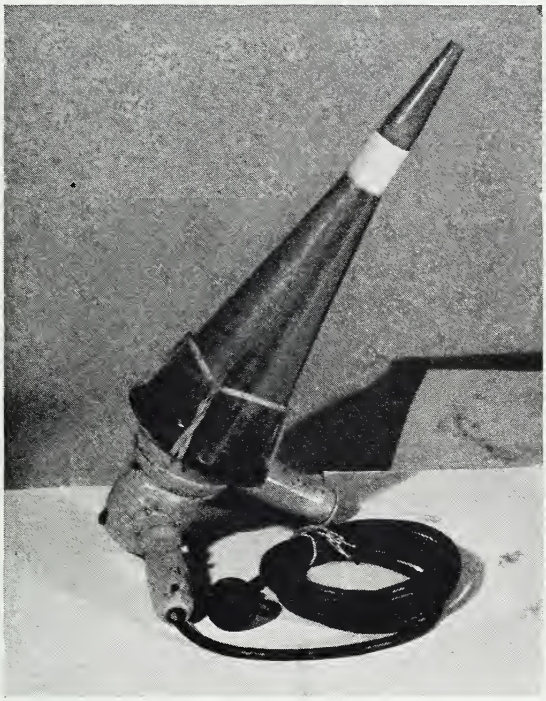

FIGURE 9.—Cone parasite collector.

COLLECTING PARASITE AND HOST ADULTS

Parasite adults to be used for mating and oviposition were drawn from the emergence cage by air suction into a celluloid cone attached to a modified hair drier (fig. 9). From the cone the adults were transferred directly to the mating cage. Parasite adults to be used for colonization purposes were drawn into a similar cone and then transferred to a large cloth-covered storage cage to await re-collection for shipment. The celluloid cone used was 9 inches long with a base 4 inches in diameter covered by a screen to hold the adults in the cone. Five hundred adults could be collected before they were transferred to the large cages.

Adults of *Ephestia kuehniella* to be used for egg production were drawn directly into their oviposition cartons (fig. 1). When not wanted for egg production all moths were drawn by means of a vacuum-cleaner fan into a large can containing a small amount of liquid to retain and kill the moths (fig. 10). The intake nozzle of this collector was of the fishtail type, and large numbers of moths could be collected in a short time. All debris, such as spilled flour, moth wings and legs, pupal cases, etc., was cleaned from the cages daily with this device.

FIGURE 10.—Collector for cleaning cages and destroying surplus host moths.

The adults of *Chelonus annulipes* are positively phototropic to a high degree, whereas the host adults remain quiescent in the presence of light. These characteristics were used to advantage in devising a collection technique. About 6 inches behind the emergence cages were placed two 18-inch long-filament lamps (fig. 11), fitted horizontally end to end and mounted on an adjustable standard so that when set up a light source of uniform intensity extended for a distance of 36 inches along the center line of the back of the emergence cage. The current for these lights passed through a rheostat so that a dim light could be used at the beginning of the collections, when large numbers of adults were present in the cages. Unless the intensity of the light was diminished by use of the rheostat the adults (including those in unopened cages awaiting collection) con-

gregated at a point nearest the light source and injured one another by their overactivity and crowding. As the numbers were diminished by collecting the intensity of the light was increased so as to draw to the rear all stragglers from the top and sides of the cage and from within the emergence boxes.

FIGURE 11.—Long-filament lamps used during parasite collecting.

The flour moth adults, for the most part, remained inactive when the light was turned on. They were collected daily only from the sides, tops, and bottoms of the cages, where smooth surfaces permitted rapid collection. None were taken from the irregular surfaces of the stacks of rearing boxes. This, of course, left a considerable number of moths uncollected in the cages each day. Therefore there was no assurance that all the moths taken in the daily collections for oviposition stock had emerged during the preceding 24 hours. This possibly accounts for the fact that the average

number of eggs per female was lower than that obtained by other workers. However, since the desired number of host eggs was always easily obtained, it was not considered practical, when collections were being made, to spend the extra time and labor necessary to produce the maximum number of eggs per host female.

MATING OF PARASITES

The parasite adults reserved for mating were stored in the mating cage in darkness at a temperature of 60° F. To induce mating, the cage was brought from the storage room and placed on a table with the rear of the cage toward a window (fig. 12). Mating began as

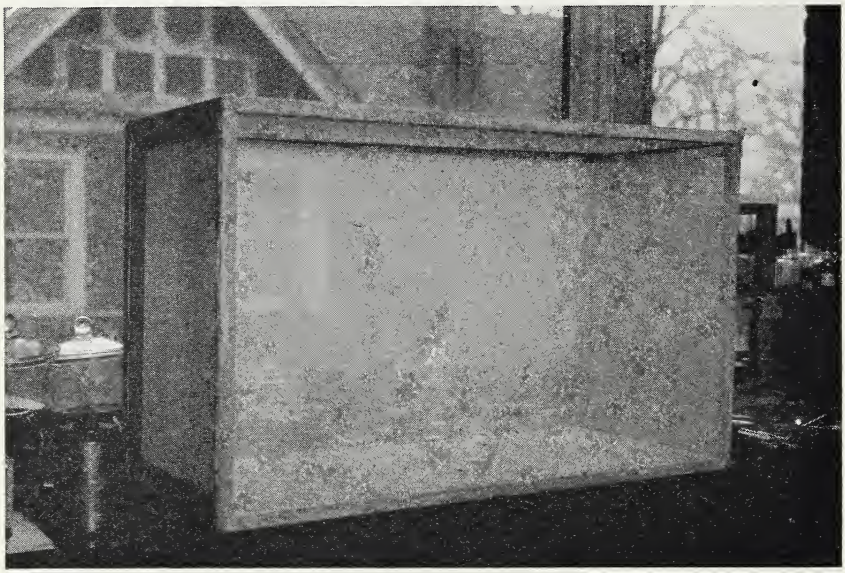

FIGURE 12.—Mating cage against the laboratory window.

soon as the light and warmth (temperatures between 74° and 80° were optimum) caused the adults to become active. Mating pairs were watched closely, and as soon as they had separated the female was brushed into one of the 3-inch oviposition vials. Thus all the females used were known to have mated. The proportion of the sexes resulting was noted in one generation only, and in this the female progeny was 51.72 percent. Since the method of mating was similar for all subsequent generations, it was assumed that the sex proportions were satisfactory. Mating was obtained with difficulty during the winter months, but in the spring it took place readily.

Food and water were supplied to adults in the mating cage only during the mating period, and this apparently was of considerable aid when the adults were slow to mate. When water was sprayed on the cloth back of the cage and large drops of concentrated sugar water were applied to the cloth with a brush, the females were induced to drink and feed. While they were feeding, the females resisted the males less violently, and much pairing was thus obtained

when other methods were unsuccessful. The procedure to induce mating was continued until all available females had mated or until the desired number had been obtained. The mating cage was then closed and returned to its storage place.

Best results were obtained by confining both sexes together continuously in the mating cage and storing it in a cool, dark place until ready for use in mating. When the cage and its contents were brought into the light and warmth, more matings were obtained and mating continued over a longer period than when the sexes were segregated between mating periods. When this technique was utilized, the females received a minimum of handling, being transferred from one container to another only three times after emergence.

REARING PARASITES FOR RELEASE IN 1938

With one exception, the locations at which releases of *Chelonus annulipes* were desired were in the eastern area of corn borer infestation and extended from northern Vermont to southern Maryland (fig. 13). Within this range the yearly generations of the borer ranged from 1 in the North to 3 in the South, with all consequent intermediate variations in time of occurrence of host eggs. The plans called for the release of 1,000 adults at each of 26 points in this area. A breeding-and-shipping schedule was therefore designed to provide enough parasite adults for liberation in the different localities on the dates when maximum numbers of host eggs would be present. Thus it was planned to ship 26,000 adults in 10 shipments, ranging from 1,000 to 7,000 adults per shipment, between May 14 and July 1.

The uncontrollable variation in the rate of development of individuals of a species having as long a life cycle as that of *Chelonus annulipes* obviously precluded any attempt to produce a given number of adults on any specific day and to produce none on days when none were to be shipped. It was also undesirable to hold individuals over for any considerable period until the number required for shipping on any day had accumulated. Observations had shown that retardation while in the developmental stage lowered production as much as 30 percent. It was therefore necessary to plan for continuous production throughout the release period, the peak of production occurring on June 9, when the greatest number would be needed. The period of development from egg to adult in the only generation reared experimentally during a period nearest this time of year averaged 48 days. It was therefore assumed that the center of the period during which parasite eggs were being laid for the production of adults for release should fall on April 22 if there was to be the optimum synchronization of parasite release with occurrence of host eggs in the field. This synchronization was accomplished during the breeding program.

Although 26,000 adults were needed for release in 1938, it was estimated that, owing to the intervals between shipping dates, about twice as many should be produced as were needed. To estimate the number of 100-host-egg clusters that should be parasitized to produce 52,000 adults, the average number of parasite adults per egg cluster produced during the rearing of 4 experimental generations in 1937

was utilized. This number ranged between a minimum of 6.4 and a maximum of 13.8, with an average of 9.5.

The variation in the average number of adults produced per cluster is due to changes in rearing technique made during the experimental period. The production of 52,000 parasite adults from host eggs

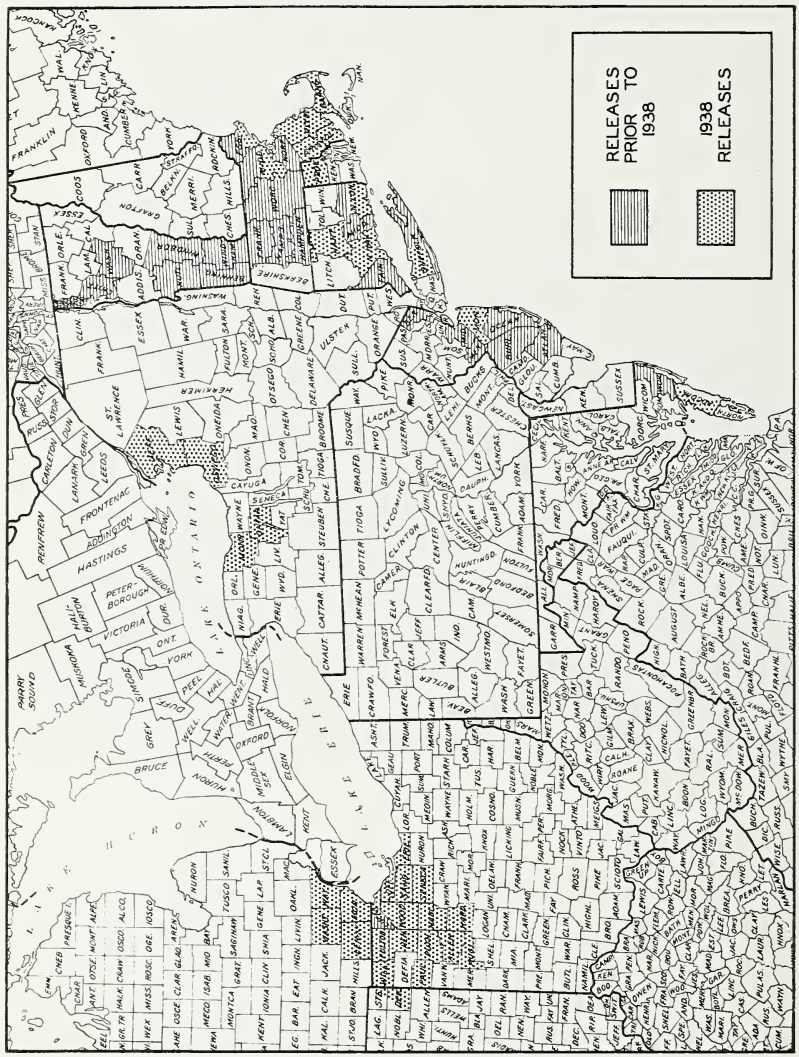

FIGURE 13.—Map showing colonization of *Chelonus annulipes*.

averaging 9.5 parasites per cluster would require a total of 5,474 parasitized host egg clusters. Actually, the total number of female-oviposition days was 5,454, so a like number of egg clusters was required.

During the oviposition period the average number of females ovipositing per day was 170.4. Thus an average of 17,040 host eggs were utilized per day. The maximum number used on any day was 38,300.

and over half a million host eggs were used during the rearing season.

Emergence began on May 6 and ended on June 25. Thus adults emerged over a period of 51 days as a result of an oviposition period that had covered 33 days. The peak which was planned for June 9 actually occurred on June 8, although the date of 50-percent emergence occurred on June 6.

In all, 133,424 adults were produced, or an average for the entire period of 2,616 per day. The maximum number produced on any day was 6,852. Since 5,454 host egg clusters were used, the number of adult parasites produced per host egg cluster was 24.5. This was over 2½ times the average used in estimating the number of egg clusters to be parasitized, and as a consequence over 2½ times the estimated number of adults were produced. The increase no doubt resulted from the accumulated effect of all improvements in rearing technique developed during the experimental period and put into effect for the generation of parasites being reared for release. The average number of adults produced per female was 783, or an increase of nearly 400-fold.

It was stated previously that a lower humidity might favor parasite development. This was indicated by the considerable variation in the number of adults produced per egg cluster in the different cages during the rearing period. Environmental and other factors were nearly identical in all cases, with the exception of the relative humidity experienced by individuals in cages installed at different periods. The cages were numbered in order from 1 to 5 as the rearing boxes were placed in them. The production of adults per egg cluster was as follows in the various cages: Cage 1, 18.4; cage 2, 30.5; cage 3, 26.0; cage 4, 23.3; cage 5, 24.5.

Individuals in cage 1 were subjected more than the others to high humidity associated with other activities being carried on in the breeding incubator. The material in cage 2 was favored, more than the others, by relatively low humidities (70 to 75 percent). Cage 4, probably more than any other, was affected by the high humidity resulting when the maximum number of *Ephestia* larvae were developing.

After production to obtain adults for release had begun, the dates of the shipping schedule were advanced 1 week because of the apparent earliness of the season. The great number of adults produced in excess of requirements, however, permitted shipments out of Toledo exactly according to the new schedule.

The production of adults in excess of those needed for release against the corn borer permitted a number of shipments for tests on the sugarcane borer (*Diatraea saccharalis* (F.)) in Louisiana, Florida, and Puerto Rico. A total of 74,000 adults was used for this purpose in June.

Parasites to be utilized for colonization were transferred from the collecting cone into large cloth-covered storage cages somewhat similar to the mating cage shown in figure 12. This permitted the feeding of large numbers of individuals in a minimum of time. A small quantity of granulated sugar was sifted evenly over the top of the cage, which was then sprayed with a fine mist just sufficient to penetrate the layer of cloth. When the cage was held in darkness at about 58° F., parasites could be retained for over 2 weeks.

Since adults of this parasite mate very readily when released in the field, no special effort was made to obtain in the laboratory the mating of those individuals intended for shipment. Much mating, however, occurred during the collection, transfer, and storage periods.

On shipping dates the storage cages were opened at a window in a room at approximately 70° F., with the back of the cage toward the light. The parasites were drawn directly into shipping cans by

FIGURE 14.—Collector and cans used for shipping adult parasites.

the use of a modified hair drier (fig. 14). When collections were made directly from the emergence cages it was difficult to prevent host adults from entering the cones. The transfer to, and recollection from, storage cages had the advantage of permitting clean colonization material for shipment to Puerto Rico where *Ephestia kuehniella* has not been recorded up to the present time.

SHIPMENTS OF PARASITES

The shipping box used most successfully for transportation of parasites has been described in detail by Bradley.[12] In previous years this

[12] BRADLEY, W. G. A THERMALLY INSULATED UNIT FOR THE TRANSPORTATION OF ADULT INSECT PARASITES. U. S. Bur. Ent. and Plant Quar. Cir. ET–77, 2 pp., illus. 1936. [Mimeographed.]

box was used for movement of material only within a 24-hour radius of the shipping point. In 1938, through cooperation with the railway express company, provision was made for re-icing en route so that at no time during transportation would a period of more than 24 hours elapse without icing. This departure from the customary routine proved highly advantageous, and a mortality of less than 0.5 percent occurred among the adults transported in this manner, some of which were in transit for as long as 36 hours. Where transportation involved shipment by rail and boat, as for the Puerto Rico material, re-icing precautions would apply only to the rail shipment, as the parasites are held under refrigeration while on board ship.

An account of the various shipping methods used and details of the colonization of *Chelonus annulipes* material reared at Toledo, Ohio, in 1938 has been presented by Clark and Bradley.[13]

SUMMARY

Chelonus annulipes Wesm. is a braconid parasite of the European corn borer (*Pyrausta nubilalis* (Hbn.)) native to northern Italy and first imported into the United States in 1929. The female deposits her egg in that of the host. The parasite larva passes through at least three instars within the host larva. During the last instar the parasite larva issues from the host, feeds externally until all remaining contents of the latter are devoured, and spins a cocoon.

After evidence had been obtained of the successful establishment of *Chelonus annulipes* near Dighton, Mass., an attempt was made to breed the parasite on the Mediterranean flour moth (*Ephestia kuehniella* Zell.) in order to provide adults for further distribution in the United States.

Host eggs were obtained by confining flour moth adults in ½-pint waxed, cylindrical, cardboard food cartons, provided with a screened bottom through which the host eggs were laid in fine-bolted white flour.

Host adults would lay eggs when no flour was present, but under such conditions only about half as many eggs were laid as when flour was provided. The absence of flour caused a slight delay in egg deposition, but this delay, providing a longer mating period prior to oviposition, was not reflected in higher egg fertility.

Host adults were collected at random from the emergence cages. There was no significant difference, either in quantity or viability, between eggs laid by female moths thus collected and those collected in the act of mating.

A maximum number of eggs was produced per carton when 500 moths were confined in each. After being stocked, each carton was used 4 days, after which the contents were discarded and all parts of the oviposition cartons were sterilized.

Special care and consideration were given all breeding operations so that invasion by mites or disease would be prevented.

Fresh flour was provided the ovipositing host females every 24 hours.

[13] CLARK, CHARLES A., and BRADLEY, W. G. DISTRIBUTION AND COLONIZATION OF EUROPEAN CORN BORER PARASITES IN 1938. U. S. Dept. Agr. Insect Pest Survey 18: 629–637, illus. 1938. [Mimeographed.]

The host eggs were separated from the flour by brushing them over an 80-mesh screen in a funnel through which a controlled current of air was drawn by a modified vacuum cleaner.

The cleaned eggs were distributed through a metal pattern over a moistened card to form circular clusters containing about 100 eggs each. The eggs adhered to the card after it had dried.

Mated parasite females were isolated during the entire oviposition period in 3- by 1-inch cork-stoppered shell vials. Food was supplied in the form of a drop of agar-sugar on a piece of paper pinned to the inner end of the cork. One host-egg cluster was put at the bottom of each oviposition vial each day and the vial was laid on its side with the bottom toward the light until parasitization was completed. When oviposition was not desired, the females were stored in a cool, dark place.

Host larvae were reared in shallow cardboard boxes measuring 6 by 6 by 1 inches with 120 cc. of whole-wheat flour spread in a layer one-fourth of an inch deep over the bottom of each box to serve for food. Fifteen egg clusters were utilized in each box. Rearing of host larvae was accomplished in an incubator room controlled to hold the temperature at 80° F. and to keep the relative humidity from falling below 70 percent.

Large numbers of growing host larvae held the humidity in the incubator room considerably above that on the outside.

The life cycle of *Chelonus annulipes* averaged 44.85 days when reared on *Ephestia kuehniella* at 80° F. in a high relative humidity. The life cycle of the host was about the same as that of the parasite.

Parasite adults were collected by being drawn by air currents into a celluloid cone attached to a modified hair drier.

Host adults to be used for oviposition were drawn directly into their oviposition cages. Superfluous moths and debris were sucked into a killing can in the intake air line of a vacuum cleaner.

The positive phototropic response of the parasite adults was made use of when collections were being made. Light caused the host moths to remain quiet.

Parasite adults were mated in a large cloth-covered cage placed with its back toward a window. All females used for parasitization were known to have mated. Unmated adults were confined, with the sexes unsegregated, in a cool, dark place between mating periods.

In the generation reared for release in 1938, 24.5 adult parasites were produced per egg cluster. The average number of females ovipositing per day was 170.4, and an average of 17,040 host eggs were used each day. A total of 133,424 adult parasites were produced during the rearing period. The maximum number emerging on any day was 6,852. The average number of adults produced per parasite female utilized was 783.

The most successful shipments of adult parasites were made in insulated boxes transported via railway express.

For shipments requiring over 24 hours of travel en route the boxes were re-iced in transit, with highly successful results.

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Division of Cereal and Forage Insect Investigations	C. M. PACKARD, *Principal Entomologist, in Charge.*

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